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ACTIVE IMMUNITY TO SYSTEMIC PLAGUE INFECTION

STUDIES IN PLAGUE, IV

FREDERICK EBERSON

North Manchurian Plague Prevention Service, Harbin, Manchuria

The problem of plague immunity has failed to lend itself to experimental methods such as have yielded successful, or at least hopeful, results in other bacterial infections.

Our knowledge concerning the mechanism of infection has been increased considerably through the recent studies of anaphylactic phenomena, particularly those dealing with the part played by certain poisons which are produced in the animal body when particular bacteria are brought into contact with substances in the serum. Friedberger's work on anaphylatoxic phenomena, as amplified by Zinsser, suggests the possibility of applying certain principles to infections which have baffled all efforts at obtaining immunity or resistance. One of the latest ideas, that of anaphylatoxins or 'proteotoxins,' broadly speaking, has thrown new light on the mechanism of bacterial infection. The whole problem of adapting these principles to specific bacterial invasions leads to questions like the following:

Does the organism, in any case, play a specific rôle in the liberation of toxic substances known as proteotoxins?

Would resistance be established for the particular organism used, if such poisons were employed to build up a proteotoxic immunity?

As to the specificity of proteotoxins, there is much to indicate that similar toxic products may be obtained by nonspecific methods, yet these results, perhaps, serve to give a broader significance to the biologic principles involved and do not detract from the importance of a mechanism which may prevail during an infection. I have stated elsewhere[1] my opinion of specificity with regard to plague. So far as the plague bacilli are concerned, I believe that they may act as a matrix for an anaphylatoxic substance and that proteotoxin obtained with B. pestis is an example of specific poison production. If this poison is specific, then resistance, when developed against it, logically ought to

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[1] Jour. Infect. Dis., 1917, 20, p. 180.

be of value in warding off the particular organism which was utilized in its manufacture. Zinsser[2] has made the first step toward demonstrating actual resistance to bacterial proteotoxins, and it seemed to me that no method could be better for studies in plague immunity than one which takes into account active bodily processes approximating the supposed mechanism of bacterial infection. This appeared to be an ideal method of approach because the evidence indicates that the mechanism of infectious disease is logically referable to some process by which toxic substances are liberated in the effort of a normal body to combat the invasive element, be it typhoid or plague bacilli. The introduction gives rise in the body to such response that immune substances may be assumed to circulate in the blood. These substances may or may not hold the balance of power, depending on the relationship between the quantity of immune-body (sensitizer), the rate of multiplication of bacteria, and the normal blood components. In the method which is about to be described, we have essentially a procedure which simulates the disease itself — the plague organisms playing an active rôle in the production of a poison which will immunize against the matrix. Obviously, the more toxic the poison, the better the chances of obtaining immunity, provided the animal may be made to survive the poison. In this manner we approximate the ideal—living virulent bacteria as immunizing agents—which in the case of plague, of course, is out of the question.

Whether or not the specificity of proteotoxin is a fact, if only for the plague organism, is not within the province of this paper to discuss. It is my contention that shock-producing substances, in all respects confirming to what we understand as 'proteotoxins,' seem to give rise to a definite resistance and immunity to the particular organism used in the experiments.

The close relationship of the phenomenon of antianaphylaxis and that of resistance to proteotoxin suggested the feasibility of applying the facts concerning the former to a method involving apparently similar principles. In an early work by Otto[3] on anaphylactic phenomena, he showed that repeated injections of considerable amounts of protein at short intervals resulted in a state of antianaphylaxis or immunity to later injections. It seemed reasonable, therefore, to assume that repeated injections of proteotoxin, if this contained specific substances of the plague organism, would give rise to an immunity toward plague itself.

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[2] Jour. Exp. Med., 1914, 20, p. 387.

[3] Cited by Zinsser, Infection and Resistance, 1916, p. 362.

The results presented here are offered hesitatingly, but I feel that in spite of the limited number of experiments, the results sems to be encouraging. Until extensive data is procurable on the value of this method for man, little can be vouchsafed as to its practicability. Experiments are now being conducted on the duration of active and passive immunity; on concentration of protective substance for both active and passive treatment; on protective and curative value of serum against virulent B. pestis over varying intervals of time; and on the potency of serum when inoculated simultaneously with B. pestis; all with the hope that the final product may be utilized in those countries where plague is sufficiently prevalent to secure convincing data as to the value of this active immunity.

TECHNIC

The materials used in the production of proteotoxins were B. pestis, antipest serum from rabbits injected with plague bacteria, and normal horse serum. B. pestis was grown on plain agar slants, incubated at from 30 to 35 C. for 24 hours. The slants were of approximately uniform size, and experience with the inoculation of cultures made it possible to attain considerable accuracy for the purpose. The proportions of culture, sensitizer and normal serum were carefully worked out as discussed in earlier publications,[4] and in the following experiments the most favorable combination was utilized.

Sensitizer was obtained according to the following method: Healthy rabbits, in weight between 1200 and 1600 gm., were injected with plague bacteria emulsified in salt solution and heated for 30 minutes at 58 C. At first, intravenous injections were made, but it was found that intraperitoneal inoculation was attended with less danger of sudden death due to agglutination in the capillaries, and likewise was capable of producing a serum potent enough for the purpose. In addition, this method of injection seemed to be more easily borne by the animals. The amount of culture, given at weekly intervals, was gradually increased from 0.1 of a slant up to a whole slant, the dosage usually being 0.2, 0.4, 0.6 of a slant for the 2nd, 3rd and 4th inoculations. At times, this treatment was modified according to the reaction of the animal so that a whole slant was injected 1 week after the 3rd dose of 0.4 or 0.5 of a culture. On an average the rabbits received but 4 doses. The criterion was always serum so obtained, which was measured macroscopically for its agglutinating power. This seemed rather remarkable in its rapidity, for when the bacterial suspension received the heated sensitizer, an almost immediate flocculation ensued, and within a few minutes the bacteria formed minute clumps in a clear fluid. Within 30 minutes at body temperature, the organisms always settled to the bottom of the tube, leaving a clear fluid above.

Horse serum was obtained with sterile precautions from the jugular vein of a normal horse, collected in a tall jar and allowed to stand in a cold room for from 18 to 36 hours. The clear serum was then pipetted off and placed in amber-colored glass-stoppered bottles. During the warmer season phenol was added as a preservative in the concentration of 0.3% but usually nothing was added to the serum which was unheated. In the preparation of proteotoxin no serum was used which exceeded 8 weeks in age.

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[4] Jour. Infect. Dis., 1917, 21, p. 56.

Proteotoxin. Agar slants of B. pestis were washed off with 0.25 c.c. NaCl solution and sensitizer, heated at 56 C. for 30 minutes, was added to the emulsion in the proportion of 1.5 to 2.0 c.c. for each 1.5 slants. After 1 hour's incubation at 37 C., the material was centrifugated for 10-15 minutes, the supernatant fluid pipetted off and the bacterial sediment washed once with salt solution. Normal horse serum was then added to the sensitized complex in amounts of 10 c.c. for each 1.5 slants of B. pestis used. The final mixture was placed in large centrifuge tubes and incubated for 14 hours at 35 to 37 C. At the end of this time, the material was centrifugated for 1 hour at moderate speed. The supernatant fluid when pipetted off was clear and gave no turbidity on agitation. Tests for the presence of B. pestis were invariably negative when the centrifugation was carried out for this length of time. Prior to injection the clear fluid was heated at 56 C. for 30 minutes.

This procedure for production of toxic substance was adopted as a standard but it was impossible to adhere rigidly to the outline, particularly in regard to the temperature of incubation during the long period when normal horse serum acted on the sensitized organisms. The intensely cold weather disarranged the incubator so that at times 27 C. was the maximum temperature recorded during the entire period of incubation. Experiments which have been described elsewhere[4] show that the temperature range is quite flexible for obtaining potent proteotoxins, so that the final product is hardly affected by variations within certain limits. Deviations with respect to the sensitizer used have been previously mentioned.

Except where otherwise indicated, the intraperitoneal method of injection was used. The absorption by this route seemed to be slower and in the amount given, devoid of shock, which was usually so severe in the case of intravenously injected (marginal vein) animals that death supervened within a few minutes. Very often the rabbits thus treated died within a day or two, or at the most after a week, with severe emaciation and slow poisoning. Intraperitoneally administered, the proteotoxin caused no visible disturbance in the animal other than a slight uneasiness at first. Ten to eleven days after the last injection with proteotoxin, virulent B. pestis was administered.

The greater part of this work was carried on at the Peiyang Medical College, Tientsin, China. I wish to thank the authorities and the Director, Dr. King, for the aid which was given, and Dr. J. R. Shand, U. S. Army, for abundant serum for the experiments.

RESULTS OF EXPERIMENTS

Exper. 1.—In this experiment to determine the value of repeated doses of plague proteotoxin in establishing a resistance to living virulent B. pestis intraperitoneally injected, the amount of sensitizer was 2.0 c.c. for each 3 slants of B. pestis and the normal horse serum added was 25 c.c. for the mixture. Preliminary tests showed that the proteotoxin was weak and could be tolerated in fairly large amounts when injected directly into the circulation via the marginal vein. The temperature of incubation during 1 hour of sensitization was 34 C., and that during proteotoxin formation was 30 to 34 C.

In this experiment it is interesting that 1 animal received considerably more proteotoxin than the 2nd, yet protection was just as marked in the latter.

Rabbit 2 showed a poor reaction to the 1st injection and continued loss in weight at each weekly examination made it imperative to discontinue treatment until more than 3 weeks later when the 2nd injection was given. The fact that this animal showed a striking tolerance to the inoculation with plague bacilli, suggested experiments with fewer doses of proteotoxin administered after longer intervals (see Exper. 5).

TABLE 1

RESULTS OF TESTS TO DETERMINE THE VALUE OF REPEATED DOSES OF PLAGUE PROTEOTOXIN IN ESTABLISHING PROTECTION

Rabbit	Weight, gm.	Dose, c.c.	Route	Dose of Pest Bacilli	Result
1	1085	6.0	Intravenous	1/200 slant, 11 days after last injection of proteotoxin	Well; lived
	1052	3.0	Intraperitoneal		
	950	2.0	Intraperitoneal		
	990	3.0	Intraperitoneal		
	1025	...	Intraperitoneal		
2	1080	5.4	Intravenous	1/200 slant, 11 days after last injection of proteotoxin	Well; lived
	910	3.0	Intraperitoneal		
	870	...	Intraperitoneal		
3	1000	...	Intraperitoneal	1/200 slant	Died after 72 hours with acute septicemic plague; all organs and blood showed enormous numbers of B. pestis
4	1200	...	Intraperitoneal	1/200 slant	Died after 50 hours; postmortem as in 3

TABLE 2

RESULTS OF TESTS MADE IN STUDYING THE TOLERANCE EXHIBITED FOR LARGER DOSES OF VIRULENT PLAGUE BACILLI

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
5	1090	7.2	Intravenous	1/100 slant	Well; lived
	1150	4.0	Intraperitoneal		
	1050	3.0	Intraperitoneal		
	1150	5.0	Intraperitoneal		
	1175	...	Intraperitoneal		
6	1015	2.8	Intravenous	1/100 slant	Died after 4 days and 6 hours; postmortem gave no typical signs of plague at the site of inoculation, the subcutaneous tissues or in the organs; slight congestion of the inguinal vessels was present; spleen smears showed numerous plague bacilli; blood smears were negative; peritoneum contained a large amount of exudate
	945	4.0	Intraperitoneal		
	860	3.0	Intraperitoneal		
	995	3.0	Intraperitoneal		
	1015	...	Intraperitoneal		
7	1420	...	Intraperitoneal	1/100 slant	Death after 80 hours; acute plague
8	1195	...	Intraperitoneal	1/100 slant	Death after 44 hours; typical plague infection

Rabbit 1 was killed 10 days after the test dose of plague bacilli was given, and at necropsy the liver showed a few minute fatty areas. Blood smears showed many polymorphonuclear leukocytes.

Exper. 2.—The object was to study the tolerance exhibited for considerably larger doses of virulent plague bacilli. Proteotoxin was prepared as in Exper. 1 and injections made at weekly intervals, with the dose of plague bacilli administered 11 days after the last injection.

Marked protection is evidenced here, as in Exper. 1. That the amount of material injected may have a bearing on the result is suggested, when the 2 animals are compared. Rabbit 6 lived about 1 day longer than the heaviest of the control animals, of which the weight exceeded that of the test rabbit by 400 gm. The findings after death indicated some degree of protection. Rabbit 5 was killed 9 days after it had received the plague inoculation. No lesions were demonstrable and blood smears showed enormous numbers of polymorphonuclears.

TABLE 3

RESULTS OF TESTS IN STUDYING THE TOLERANCE EXHIBITED FOR LARGER DOSES OF VIRULENT BACILLI AT A DIFFERENT INCUBATION TEMPERATURE

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
9	1065	2.0	Intravenous	1/100 slant, 10 days after last injection	Well; lived
	1115	4.0	Intraperitoneal		
	1075	3.0	Intraperitoneal		
	1025	3.0	Intraperitoneal		
	1105	4.0	Intraperitoneal		
	1175	...	Intraperitoneal		
10	1165	4.8*	Intravenous	3/200 slant, 10 days after last injection	Death in 5 day and 9 hours there wer chronic change in liver an spleen
	1125	3.0	Intraperitoneal		
	1010	2.0	Intraperitoneal		
	1157	4.0	Intraperitoneal		
	1175	...	Intraperitoneal		

* Severe shock followed the injection of proteotoxin. As controls two rabbits weighing respectively 1,200 and 1,190 gm., were given 1/100 slant intraperitoneally. These animals died within 3 days of acute septicemic plague.

Exper. 3.—The procedure was the same as in Exper. 2, with the exception that the incubation temperature during the 14-hour period ranged from 31 to 33 C. and an additional injection was made in Animal 9.

Here we see again a definite resistance on the part of treated animals. Even 3/200 of a slant, which is far in excess of a "minimal lethal" dose, failed to kill Rabbit 10 within the usual time, which for this dose would be less than 40 hours. This animal appeared perfectly healthy until the 4th day after inoculation when it became apathetic and refused food. Rabbit 9 was etherized 7 weeks after receiving the dose of B. pestis. No changes were noted in the organs. The serum was used as sensitizer in other experiments.

Exper. 4.—It seemed of interest to study the action of filtered proteotoxins in an effort to correlate shock production with the development of tolerance to living bacteria. Some writers have found that filtration of proteotoxin inhibits shock, and an experiment was made with the hope that a series of injections with filtered material would reveal an interesting relationship. The results of Zinsser,[5] with regard to the peculiar behavior of proteotoxins were confirmed. A series of animals which were injected intravenously with considerable quantities of the material showed no shock, whereas controls which were treated with unfiltered material reacted severely in typical fashion. Unfortunately, it was possible to make but one injection with such filtered proteotoxin—the 1st dose—on account of an accident. The protocol serves, therefore, as an additional experiment similar to those which precede.

TABLE 4

RESULTS OF TESTS MADE TO DETERMINE THE ACTION OF FILTERED PROTEOTOXINS

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
13	610	2.8*	Intravenous	1/400 slant, 10 days after last injection	Well; lived
	557	3.0	Intraperitoneal		
	548	3.0	Intraperitoneal		
	620	3.8	Intraperitoneal		
	725	...	Intraperitoneal		
14	595	2.6*	Intravenous	Same as 13	Well; lived
	695	3.2	Intraperitoneal		
	695	3.0	Intraperitoneal		
	715	4.0	Intraperitoneal		
	765	...	Intraperitoneal		
15	620	3.0	Intraperitoneal	1/200 slant, 10 days after last injection	Death in 48 hours; acute plague
	580	1.8	Intraperitoneal		
	595	2.5	Intraperitoneal		
	630	3.5	Intraperitoneal		
	700	...	Intraperitoneal		
16	695	3.2	Intraperitoneal	1/100 slant, 10 days after last injection	Death, acute plague, after 44 hours
	700	3.5	Intraperitoneal		
	765	4.0	Intraperitoneal		
	740	4.0	Intraperitoneal		
	795	...	Intraperitoneal		
17	945	...	Intraperitoneal	1/200 slant	Death, acute plague, after 60 hours
18	940	...	Intraperitoneal	1/400 slant	Death, acute plague, after 80 hours

* Proteotoxin passed through Reichel filter.

Rabbits 15 and 16 became ill within 24 hours after the test dose. Necropsy revealed a severe infection. With regard to virulent B. pestis, it is impossible to draw a sharp line of demarcation between a "minimal lethal" dose and one which, if resisted, would tend to illustrate the point desired. Considerable doses were purposely given in this series of small animals in order to test the resistance toward an exaggerated infection. The animals naturally differ in their individual susceptibilities to inoculation, but it may be said that for plague, the natural mode of infection requires but a very slight amount of material which need not be introduced directly into the system as has been done in all of these experiments. Detailed studies dealing with the relation between the total amount of proteotoxin given and the resistance to varying doses of B. pestis would make this point clear.

Exper. 5.—I have mentioned that 2 injections of proteotoxin, with a considerable interval, seemed to protect a rabbit so treated. In order to study the value of such a procedure, a series of animals were given 2 injections of the material allowing 2 weeks to elapse between the doses. Proteotoxin was prepared in the following manner: Three slants of B. pestis were washed off with 0.75 c.c. NaCl solution and 4.0 c.c. of sensitizer added. After incubating for 1 hour at 37.5 C. the mixture was centrifugated and the sediment recovered and washed once with salt solution. Twenty cubic centimeters of normal horse serum were added to the complex and the whole incubated at 37.5 C. for 14 hours. One hour's centrifugation rendered the supernatant fluid entirely clear. This was heated for 35 minutes at 56.5 C. and injected intraperitoneally.

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[5] Jour. Exp. Med., 1914, 20, p. 387 and p. 582.

TABLE 5

RESULTS OF TESTS MADE TO DETERMINE THE PROTECTIVE VALUE OF INJECTING THE PROTEOTOXIN AT INTERVALS OF 2 WEEKS

Rabbit	Weight, in gm.	Dose, in c.c.	Dose of Pest Bacilli	Result
19	955 1012 1000	3.0 4.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived
20	950 980 995	3.0 3.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived
21	820 715 815	3.0 3.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived

Controls

Rabbit	Weight, in gm.	Dose, in c.c.	Dose of Pest Bacilli	Result
22	975	...	1/400 slant, intraperitoneally	Death in 5 days; acute plague
23	910	...	1/400 slant, intraperitoneally	Death in 6 days; acute plague
24	1015	...	1/400 slant, intraperitoneally	Death in 6 days; acute plague

The results indicate that a definite response is elicited in the animal body by so few as 2 injections 2 weeks apart. This interval is of distinct advantage in enabling the animal to stand up under the strain of treatment which normally occurs. One of the primary objects of this experiment was to attempt to throw some light on the question of what influence a few heated sensitized B. pestis may have in bringing about the resistance noted. A small total volume of material was given so that in the amount of proteotoxin injected the few organisms which might be present would hardly have a significant effect. In order to double-control this point, which appears to be of theoretical interest, at least, the following experiment was made.

Exper. 6.—Six slants of B. pestis were sensitized for 1 hour at 37.5 C. with 8.0 c.c. of serum, centrifugated to remove the organisms, and the sediment washed once with normal salt solution. To the organisms were then added 40 c.c. of normal salt solution and the suspension finally incubated at body temperature for 14 hours. After an hour's centrifugation, the supernatant fluid was pipetted off and heated at 56.5 C. for 30 minutes. Injections were made into the peritoneum at weekly intervals, as in the proteotoxin experiments, and living virulent plague bacilli injected via the same route 10 days after the last dose of test substance. The material which was used for this experiment appeared fairly clear after prolonged centrifugation and, in 1 instance, was distinctly turbid with B. pestis.

TABLE 6

TESTS MADE TO DETERMINE THE INFLUENCE OF HEATED SENSITIZED B. PESTIS IN BRINGING ABOUT RESISTANCE TO INFECTION

Rabbit	Weight, in gm.	Dose, c.c.	Dose of Pest Bacilli	Result
25	1360	6.0	1/200 slant	Death, acute plague, 48 hours
	1260	4.0*		
	1190	3.4		
	1182	4.0		
	1230	...		
26	720	4.0	1/400 slant	Death, acute plague, 46 hours
	650	4.0*		
	575	3.0		
	610	6.0		
	710	...		
27	930	4.0	1/400 slant	Death, acute plague, 40 hours
	845	4.0*		
	865	5.0		
	862	5.0		
	795	...		

* Centrifuge defective; supernatant fluid heavily clouded with B. pestis.

Although few animals were used in this experiment, the results seemed to be well marked, since the amount of material injected is considerably greater than that used in any of the other experiments. In addition, we have the fact, although a mere accident, that the 2nd injection was made with material which might by itself be expected to possess some immunizing action inasmuch as it contained enormous numbers of plague bacteria which were not removable by centrifugation.

In this study no attempt has been made to determine the duration of active immunity, except in a preliminary way. A series of animals which were given several injections of the material, were to be tested with doses of living virulent plague at intervals of 1, 2, 3, 4, 5, and 6 months, respectively, after treatment. Owing to discouraging mortality among the animals, the completeness of the experiment was interfered with and it is possible to report the results for those animals only which received the test dose of plague bacteria 1 month after the last injection of proteotoxin.

Exper. 7.—The rabbits in this series received 4 intraperitoneal injections of proteotoxin obtained with B. pestis. The technic followed was the same as that in preceding experiments. The test dose of plague bacilli was administered via the peritoneum 30 days after the final injection.

The results show that rabbits treated with plague proteotoxins are definitely resistant to inoculation with B. pestis at least 1 month after treatment. Two untreated animals (31 and 32) became ill on the 2nd day following injection with B. pestis and died of typical plague in about 4 days. The 3 treated rabbits showed no illness whatever and remained alive. One of these was killed 10 days after the test dose of organisms and showed no lesions. The blood contained many polymorphonuclears.

An interesting question arises as to the mechanism of this resistance to infection with living organisms used in the preparation of the proteotoxins; also, as to the localization of this apparently specific tolerance. From the scanty material available in the course of these experiments, it seems as if some support might be given to the hypothesis of a powerful leukocytic response to the substance injected. When animals were treated intraperitoneally, an injection of the test dose of plague bacteria was invariably followed by marked accumulation of polymorphonuclears in the peritoneal exudate, as well as in the blood. This was not observed in untreated rabbits. Whether or not the substance gives rise to a bacteriolytic action has not yet been determined. The question of localization of tolerance may be passed over with a few words. From the nature of plague infection it is evident that an absolute immunity toward inoculation by any particular route would depend on the state of resistance developed by the treatment. Results of tests made on these points may, therefore, fail to answer the question. There is no way of controlling the efficacy of the treatment other than by the use of a very large series of animals for each type of inoculation practiced, and by further making cross injections with plague organisms, in order to determine the value of intraperitoneal injections of proteotoxin for intravenously inoculated B. pestis and that of intravenous treatment for organisms administered by a different route. The few experiments which were made on this point indi-

TABLE 7

RESULTS OF TESTS WHEN 4 INTRAPERITONEAL INJECTIONS OF PROTEOTOXIN OBTAINED WITH B. PESTIS WERE GIVEN

Rabbit	Weight, in gm.	Dose, c.c.	Dose of Pest Bacilli	Result
28	610	2.0	1/400 slant	Well; lived
	645	3.0		
	680	4.0		
	715	4.5		
	605	...		
29	650	2.0	1/400 slant	Well; lived
	715	4.0		
	725	4.5		
	765	5.0		
	740	...		
30	625	2.5	1/400 slant	Well; lived
	640	3.0		
	635	3.0		
	650	4.0		
	710	...		
Controls				
31	810	...	1/400 slant	Death after 4 days; acute plague
32	790	...	1/400 slant	Death after 4½ days; acute plague

cate that the animal does not tolerate any considerable dose of plague when the bacteria are given intravenously after treatment with proteotoxin. Of course, since it was not possible to determine with sufficient controls whether or not the material was efficacious, it is difficult to venture an opinion.

Exper. 8.—The following table records the results of a typical experiment.

TABLE 8

RESULTS OF TESTS TO DETERMINE THE MECHANISM BY WHICH THIS RESISTANCE TO INFECTION TAKES PLACE

Rabbit	Weight, gm.	Dose, c.c.	Route	Dose of Pest Bacilli	Result
33	1115	1.0	Intravenous	1/400 slant, 11 days after last injection	Death in 5 days
	1045	3.5	Intravenous		
	980	3.0	Intravenous		
	930	2.5	Intravenous		
	965	...	Intravenous		
34	1335	3.2	Intravenous	1/400 slant, 11 days after last injection	Well; lived
	1385	4.0	Intravenous		
	1275	4.0	Intravenous		
	1444	5.0	Intravenous		
	1320	...	Intravenous		
35	1200	2.8	Intraperitoneal	1/800 slant, 10 days after last injection	Well; lived
	1197	3.8	Intraperitoneal		
	1286	4.5	Intraperitoneal		
	1026	...	Intravenous		
36	1025	3.0	Intraperitoneal	1/400 slant, 10 days after last injection	Death in 3 days; blood negative; very few organisms in spleen
	1098	3.6	Intraperitoneal		
	1172	5.0	Intraperitoneal		
	984	...	Intraperitoneal		

Controls

37	1408	...	Intravenous	1/800 slant	Death in 3 days; acute septicemic plague
38	1765	...	Intraperitoneal	1/400 slant	Death, acute plague
39*					

* This rabbit was treated with salt solution prepared as in experiment 6, as an added control. The animal received, in all, 16 c.c. of material in 4 intravenous injections, and the test dose of B. pestis was given 10 days after the last injection, into the ear vein. At each weekly injection the animal weighed respectively 1325, 1340, 1270, 1165 gm. and 1151 gm. at the time it received the plague inoculation. One eight-hundredth of a slant B. pestis killed the rabbit in 4 days.

SUMMARY AND CONCLUSIONS

The treatment of rabbits with proteotoxins obtained with B. pestis confers a definite resistance on the part of the animal to systemically introduced plague organisms.

There is apparent protection in at least 75% of the animals treated, taking the entire number of cases into consideration. Below a certain dose of bacteria, the protection conferred is much greater.

Combined intravenous and intraperitoneal injections of the proteotoxin seem to be more effective than either method by itself, although intraperitoneal treatment of rabbits results in a powerful resistance to plague bacteria administered by that route.

The immunity, so far as it has been possible to determine in these experiments, is definitely marked for at least 1 month after treatment.

Injections given at intervals of 2 to 3 weeks give rise to a definite resistance to inoculation with living virulent plague bacilli.

B. pestis appears to be the matrix for a specific poisonous substance of the nature of proteotoxin, and capable of inducing a specific resistance to the organism which is used in the manufacture of the toxic element.

Observations so far support the hypothesis that one of the most potent factors in the protective mechanism is that of leukocytosis.